To sum up the proper steps as we know them at present, in the diagnosis of rabies in animals, the following points may be noted: Any animal suspected of being rabic should, if possible, be captured alive, placed in a cage where it can do no harm and watched. If it has rabies it will die in a few days. No rabic animal ever recovers. To exclude other causes of death, the brain should be removed and an examination of its various parts made according to the methods above described for Negri bodies and incidentally for the Nelis-van Gehuchten reaction. If the bodies are found, the diagnosis may be considered absolute. This will take only a few hours. If the bodies are not found, then the inoculation test on animals should be made, which requires, as a rule, from 2 to 3 weeks. The demonstration of the bodies of hydrophobia, therefore, is of great importance to the physician in cases of persons bitten by suspected animals, for by sending the brain of the animal to a well-established laboratory an almost absolute diagnosis may be had in a few hours, and, if positive, the Pasteur treatment advised at once.

NATURE OF THE NEGRI BODIES.

Concerning the nature of these bodies, little of a definite character may be said. Negri, in his first paper, stated that he believed them to be protozoa and probably the cause of the disease. This, of course, he could not demonstrate. Volpino[4] has attempted to arrange in a cycle the various forms which he observed in the hippocampus major in dogs inoculated with street virus and killed at the first appearance of rabic symptoms. In this cycle he has 8 different steps, but this does not include all the forms he observed. It has been shown by Remlinger, Barratt, Bertarelli and others that the virus passes through the Berkefeld filter No. 5. This does not exclude the Negri bodies from being etiologically associated with the disease, for forms so small that they can hardly be seen with the highest magnification occur, and there is no reason why forms still smaller may not also exist. Volpino, in his cycle, considers one stage as that of the filterable virus.

That Negri bodies as we see them can not be considered identical with the virus seems quite clearly demonstrated. It has been shown that the hippocampus major where they are most numerous is no more virulent than other parts of the brain in which they may not be found at all. The medulla of rabic animals is considered to be the most virulent part of the nervous system, yet the bodies are often absent from this region or occur in small numbers. Bertarelli[5] also showed that the hippocampus became virulent 2 to 3 days before the appearance of Negri bodies in that region.

The possibility of these bodies being degeneration products has been carefully considered. In this respect they must pass through the same critical ordeal to which the analogous bodies of cancer, smallpox, scarlet fever and the like have been subjected. All that can be said at present is that they are different from any degeneration product or any morphologic entity, normal or pathologic, thus far known. They are specific, therefore, for this disease and, whether degeneration product or protozoa, are important as diagnostic structures.

Uremia.—Dr. S. West says that we seem driven to suppose that the symptoms of uremia are due to some substance suddenly developed in the body in some mysterious way, and it is on chemico-physiologic lines that we must seek an explanation. —*Clinical Journal*, May 30, 1906.

4. Centralb. f. Bakt., Referate 1905, xxxvii, p. 459.
5. Centralb. f. Bakt., I Abt. Orig. vol. 39, p. 399.

THE STAINING AND EXAMINATION OF THE BACTERIA OF THE EYE BY SIMPLE PRACTICAL METHODS.*

EDGAR S. THOMSON, M.D.
NEW YORK CITY.

The staining of the bacteria of the eye with a view to the practical benefits to be derived therefrom may be conveniently divided as follows: Film preparations, including both smears from fresh conjunctival discharge and culture preparations; scrapings from corneal or conjunctival ulcers; tissue preparations.

Film preparations from the fresh conjunctival discharge, which represent the greater proportion of our work at present in the Laboratory of the Manhattan Eye, Ear, and Throat Hospital, are of value not only in arriving at a correct diagnosis, but also in the study of the progress of the disease. Every case which is admitted to the contagious wards has several films made, followed by cultures if this latter seems necessary; and thereafter, as long as the patient remains in the hospital, film preparations are made every two or three days. In preparing the films some care is necessary in order to secure the best results. After thoroughly cleansing the slides in alcohol, a small amount of pus from the conjunctival cul-de-sac is taken up on a platinum wire loop and spread as thinly as possible on the slides. In doing this, the drop should be led gently over the slide rather than rubbed on, for if too much violence is used the relations of the cells and bacteria may be disturbed. The practice of taking the pus on a cotton swab and rubbing it over the slide is a very bad one and likely—in the case of gonococcus at least—to lead to erroneous results. The slides are then dried and are ready for staining.

For general work, Loeffler's methylene blue continues to be our most useful stain. It is made by adding 30 parts of concentrated alcohol solution of methylene blue to 100 parts of 1 to 10,000 aqueous solution of caustic potash. This solution is poured over the slide and rocked back and forth until the smear is colored a fairly deep blue, generally in from a few seconds to a minute. The slide is then washed freely and dried, when it is ready for examination with the oil immersion lens. No coverglass is necessary; the drop of oil is placed directly on the slide. The pus and epithelial nuclei are stained deep blue, the cytoplasm and mucus faintly, while the majority of the bacteria are stained to about the same intensity as the pus nuclei. Carbol fuchsin in 5 per cent. solution may be used in the same way, but it offers no especial advantages in the majority of cases over the methylene blue, so in practice we do not often use it.

Counter-staining is of distinct advantage in showing certain details not ordinarily visible; the cytoplasm shows more plainly, the contrast is greater, and there is better cell differentiation, showing the relation of cells to bacteria. Our pathologist, Dr. E. G. Zabriskie, uses Wright's modification of Jenner's blood stain (eosinate of methylene blue), or Goldhorn's polychrome methylene blue. In preparing the eosinate of methylene blue the first step is to precipitate the red in methylene blue by dissolving 1 gm. of methylene blue in a 0.5 per cent. solution of sodium bicarbonate. This mixture is steamed for one hour in an Arnold steam sterilizer, then cooled rapidly, and poured into a large porcelain dish. Under constant stirring, a 1 to 1000 aqueous solution of yellow eosin is then added until a metallic scum appears.

* Read in the Section on Ophthalmology of the American Medical Association, at the Fifty-seventh Annual Session, June, 1906.

About 400 or 500 c.c. of the eosin solution will be found necessary for 100 c.c. of the original methylene blue solution. The precipitate is then collected by filtration, carefully dried by gentle heat, and a saturated solution is made of the precipitate in pure methyl alcohol. About 0.3 per cent. is necessary for saturation. The mixture should then be carefully filtered, and 25 per cent. of methyl alcohol added.

In using the stain no preparatory fixation is required. The stain is applied for one minute, during which fixation takes place through the methyl alcohol. Distilled water should then be added, drop by drop, until a metallic scum forms on top of the liquid and the edges are a purplish red color. The actual staining now takes place, and this is kept up for from 3 to 5 minutes. The slide is then washed and dried and the smear examined.

The washing is a delicate process; on it depends in great part the success of the stain. It must be done very slowly, carefully and thoroughly. The nuclei will be found blue to purple green, the bacteria purplish, and the neutrophilic granules purplish pink. Goldhorn's polychrome methylene blue is used exactly as Loeffler's methylene blue. The stain may be bought already mixed, and the smear is simply stained for one minute or less, washed and dried.

Decolorization is of great value, from a diagnostic standpoint, owing to the fact that different bacteria give up the stain with very different degrees of readiness. It is especially valuable in the case of the gonococcus, and is best accomplished by the method of Gram, which is as follows:

Fix with gentle heat.
Stain with anilin gentian violet three minutes.
Wash with salt solution.
Add iodin solution for one minute.
Wash.
Decolorize with absolute alcohol until the smear looks steel-gray.

The most important germ with which we have to deal from the practical clinical standpoint—both from the severity of its effects and its relative frequency—is the gonococcus. In typical cases, soon after the onset of the disease, we find under the microscope large clusters of perfectly characteristic biscuit-shaped diplococci lying chiefly within the pus and epithelial cells and showing a pale capsule surrounding each pair of individuals. Tetrads are uncommon in the conjunctiva. They stain well with Loeffler's methylene blue or either of the counter-stains above mentioned.

In the great majority of cases, the clusters are perfectly characteristic for the first 5 to 7 days after the onset of the disease, and if the clinical appearances of gonorrheal conjunctivitis are well marked we make no further examination. If, however, the clinical appearances are not characteristic and the smears show many varying forms lying outside of the cells, we next proceed to decolorize by Gram's method. If the bacteria decolorize, we have either the gonococcus or the diplococcus of meningitis, or perhaps the *Micrococcus catarrhalis*. Modern observers almost all agree that staphylococci, though they may assume the form of diplococci, never have the concavities facing each other as in the case of the gonococcus, and the capsule—except in the pneumococcus—is also lacking. It is easy, therefore, to say that a case which has the typical appearance and in which the clinical symptoms are characteristic is gonorrheal; while it is extremely difficult to say that any given case—especially late in the disease—is not gonorrheal. The meningococcus may assume the typical form of the gonococcus, but is rather rare in the conjunctiva and causes a much less severe inflammation. Many diplococci will be found lying outside of the cells and numerous varying forms will be present. If doubt still remains, cultures alone will decide the diagnosis.

As the attack of gonorrheal conjunctivitis yields to treatment, the groups of gonococci grow smaller and show many varying forms (involution forms) of less characteristic shape, but as long as the least amount of discharge remains the germs will be found. Some writers assert that the gonococci may be found even after the discharge subsides. On this point I can not speak from actual experience, but when the pus cells disappear and the discharge assumes the catarrhal form, with mucus predominating, gonococci can not usually be demonstrated, and it is our custom to discharge the patient from the hospital wards.

The diplococcus of meningitis deserves mention, not from the frequency of its occurrence—for all agree that it is rare in the conjunctiva—but on account of its similarity to the gonococcus. It occurs within the polynuclear leucocytes, but, according to Park,[1] "it never appears within the nucleus and rarely within other cells." It is decolorized by Gram, as is the gonococcus, but the cultural differences—into which I will not enter here—are decisive. During about eight years we have seen at the Manhattan Eye, Ear and Throat Hospital only three or four well-established cases to about 400 cases of gonorrheal ophthalmia. Of course, it should be said that the severe cases do not come into the wards, and this would, no doubt, somewhat reduce the number of the meningococcus cases. Parsons[2] says: "The rarity of this organism scarcely vitiates the ordinary rules of procedure, but in cases of doubt cultivation experiments must be resorted to." The *Micrococcus catarrhalis* (R. Pfeiffer) is believed by Park to have been at times confused with the meningococcus, and it is possible, of course, that our cases were caused by this organism. The clinical symptoms were those of a very insignificant conjunctival affection, and a few days' treatment sufficed to clear up the discharge; so that, lacking the peculiar cell penetration that is so characteristic of the gonococcus, it seems fairly certain that they were either meningococci or one of its allied forms, and not the gonococci, and, as this was for us the point of practical importance, I regret to say that no careful culture studies were made. Of course, the clinical condition and history were carefully considered, as they should always be.

Other diplococci occasionally resemble gonococci, but most of them are positive to Gram. In this connection it should be remembered that, on account of the rapid onset and alarming appearance of gonorrheal ophthalmia, such cases usually come under our care early, at a time when the gonococcus is well developed and is most characteristic in smears. Later, when involution forms begin to appear, the diagnosis is more difficult and smears are of less value. Another fact which makes early smear examinations important is that the other pus cocci develop more slowly than the gonococci, and early smears show very few other organisms.

The detection of the Koch-Weeks bacillus in smears is fairly easy. It belongs to the group of influenza bacilli and seems to be the commonest cause of acute contagious conjunctivitis. The clinical characteristics of the disease caused by it vary considerably. It is worse in some epidemics than in others, so that we have

1. Park: Pathogenic Micro-organisms, p. 361.
2. "The Pathology of the Eye," vol. I, p. 44.

little to help us in the clinical symptoms. They appear as short fine rods (0.8 by 10 micromillimeters) lying frequently between the pus cells and also within the cytoplasm in great numbers. They frequently lie end to end in the shape of the letter V. They take the stain with difficulty, and in methylene blue preparations stain usually to about the intensity of the cytoplasm, so that a first glance through the microscope gives only a faint idea of the number usually present. As more careful examination is made the bacilli may be seen by hundreds. They are best stained with Ziehl's solution of carbol fuchsin, 1 part to water 9 parts, or with

According to Park, it is to be differentiated from: 1, the influenza bacillus of Pfeiffer; 2, the so-called influenza bacillus of conjunctivitis, of Müller; 3, the pseudo influenza bacillus of Zur Nedden. All these grow only on a hemoglobin medium, which is not required by the Koch-Weeks bacillus. They are nearly allied forms and can not be morphologically differentiated from the Koch-Weeks, but from a practical standpoint the film diagnosis is of considerable value, as the other forms are probably much rarer. In cultures of the Koch-Weeks, club-shaped involution forms commonly appear. It is decolorized by Gram.

The Morax-Axenfeld diplobacillus has quite a characteristic appearance in smears. It causes a low grade of chronic blepharo-conjunctivitis, and occurs plentifully in the conjunctival discharge in such cases. The bacilli are large (2 micromillimeters by 1), though this varies, and they are frequently found in chains. They stain well with ordinary anilin stains and are decolorized by Gram. According to Parsons, the *Bacillus liquefaciens* of Petit is a diplobacillus closely allied to the Morax-Axenfeld. It occurs in corneal ulcerations, especially of the corneal margin, and, as a rule, is smaller than the Morax-Axenfeld. Cultural differences are marked and will establish the diagnosis.

The Klebs-Loeffler bacillus of diphtheria, which occurs very rarely with us in the conjunctiva, is a short thick bacillus which varies considerably in size and virulence, facts which Parsons believes account for many of the so-called pseudo-diphtheria bacilli. There are no differential stains of reliability, and cultures, which are best made in Loeffler's blood serum, are invariably necessary. The cultural forms vary considerably and show a marked beading or polar staining.

The xerosis bacillus of Colomiatti closely resembles the bacillus of diphtheria, both morphologically and culturally. The whole question of differential diagnosis is an involved one. Both bacilli stain readily with the ordinary anilin dyes and are easily cultivated, and, aside from the chemical differences—which have recently been studied a good deal and seem to promise well—the subject seems to resolve itself into the degree of virulence of the individual growth. Within the past eight years we have had but four cases of genuine conjunctival diphtheria in our wards, and in these the severity of the clinical symptoms left no doubt that we were dealing with true diphtheria.

Staphylococci, streptococci and their allied forms deserve only a passing mention in the present connection. They all stain readily with anilin dyes, have no differential stains of value, and must be studied by cultures and inoculations to arrive at a correct bacterial diagnosis.

The pneumococcus of Fränkel and Weichselbaum is of importance, both as regards its rôle in conjunctivitis and in ulcus serpens of the cornea. It frequently has a characteristic shape in smears, and a diagnosis can be made with a fair degree of certainty in many instances, but in other cases the forms vary so greatly that cultures must be made. When typical, it has the shape of two cartridges placed end to end and surrounded by a pale capsule. In many instances this capsule is absent and the lanceolate shape is not marked, and it may even form short chains. As a modern writer puts it: "Its chief characteristic is variability." It is best stained with weak carbol fuchsin, as in the case of the Koch-Weeks. The slide is gently heated during the staining, in which case the capsule, if present, will show clearly.

Scrapings from a conjunctival or a corneal ulcer may frequently give valuable information. If the case is one of suspected tuberculosis of the conjunctiva, it is best, on account of the scanty distribution of the bacilli, to excise a piece of tissue from the margin of the ulcer carefully and to "shred" it in a small amount of normal salt solution, and then to spread the whole on slides which are stained for tubercle bacilli with carbol fuchsin and methylene blue, a method that is so well known as to need no description here. Corneal scrapings should be treated in the same manner and then stained with methylene blue. It is not, however, always possible to find the distinctive germ in such specimens, as every one knows who is familiar with such examinations. Even in positive cases we usually find but a few bacteria. Tissue preparations are also unsatisfactory at times and frequently in undoubted cases of infection bacteria can not be demonstrated in the sections. This is partly due to the fact that it is so difficult to get the sections thin enough for accurate bacterial study, and partly due to the early destruction of the bacteria by the phagocytes. We use in preference the toluidin blue stain, which is not difficult and at times gives good results. The various steps of this method are as follows:

Dissolve 1 per cent. of toluidin blue in 1 or 2 per cent. carbolic water.

Stain from fifteen to thirty minutes.

Decolorize in 95 per cent. alcohol, to which, if the color is given off slowly, add a few drops of glacial acetic acid.

If the acid is added it is again washed with 95 per cent. alcohol.

Clear rapidly in carbol xylol.

Immerse for a few seconds in oil of origanum.

Mount.

The proper degree of decolorization is reached when the sections show only a faint trace of the original blue color—in other words, show only a sharply defined stain of the nuclei, basophilic granules, and bacteria. If counter-staining is desired, the sections, after staining in toluidin blue, are immersed in water and then in a 0.5 per cent. solution of aqueous yellow eosin, where they are allowed to remain 10 minutes or longer. They are then transferred to 95 per cent. alcohol, where the dehydration and decolorization take place at the same time. They are then cleared and mounted. By this modification the fibrous tissues are stained pink.

Unna's method may also be used by simply substituting Unna's alkalin methyl blue for the toluidin blue. The other steps in the staining are the same.

DISCUSSION.

DR. BROWN PUSEY, Chicago, said that he thought the paper would be greatly improved if the title read, "The Staining and Examination of the Bacteria of the Eye by Accurate Methods." This point seemed the more important because recently he had been asked often for advice as to how one can quickly and easily get a working knowledge of bacteriology for use in this specialty and how quickly to make examinations of the secretion. His answer to the first question was that the matter is

so important that it deserves not quick and simple consideration, but thorough work, and to the latter that such an examination should not be a matter of quickness, but of accuracy, that it is so important in every case that abundance of time should be given to make a careful examination. In answering this question the matter of depending on the clinical conditions for determining the character of the infectious agent had come up several times, and to answer this question he had but to cite two recent cases. One in the clinical diagnosis was made by capable men of probable gonorrheal conjunctivitis; bacteriologic examination showed the diplobacillus of Morax-Axenfeld to be the pathogenic agent. Another was in an elderly woman who was caring for a case of gonorrheal conjunctivitis. On one of his visits he found this woman's eyes slightly red, and bacteriologic examination showed the presence of gonococci; the conjunctivitis resulting from this infection was so trivial, however, that the patient did not realize that anything was wrong, and she recovered without treatment. These two cases show how reckless it would be to venture an opinion of the nature of the infectious agent from clinical conditions.

Dr. Pusey emphasized the importance of cultures in bacteriologic diagnosis even more than did Dr. Thomson. He said that he had been able to make a diagnosis from cultures when smears failed, or rather when he failed to find organisms in smears. He had heard the complaint that it is difficult to keep on hand a supply of culture media. The media that one really needs is not hard to get if one makes a little effort.

As regards the frequency of occurrence of the various pathogenic organisms of the conjunctiva, his experience in Chicago differs from that of Dr. Thomson. Last year he had 14 cases of pneumococcus conjunctivitis, 13 cases of diplobacillus conjunctivitis, 4 cases of conjunctivitis due to the influenza bacillus, 3 due to the gonococcus and a few cases due to the staphylococcus. He has not yet found the Koch-Weeks bacillus in Chicago.

DR. ARNOLD KNAPP, New York, believed that the bacillus of influenza would be found more frequently in these cases if proper culture media were used. The relative importance of the smear and cultures for determining the organisms he considered a difficult question. No less an authority than Morax states that a smear is all that is necessary, that a culture of the organism in the conjunctiva is of little value and apt to be misleading. While that may be true in some cases, Dr. Knapp said it certainly is not of the diphtheria bacillus. It can not be told from the non-virulent forms simply by its morphology, and the practiced bacteriologist will not make a diagnosis simply by the character of the culture. The Board of Health of New York will not make a diagnosis of diphtheria from cultures obtained elsewhere than from the throat, without knowing the clinical manifestations of the case. By the fermentation tests in serum water media (Journal of Medical Research, vol. xii, No. 4) this differentiation can be made in a much simpler manner than by animal inoculations.

DR. GEORGE S. DIXON, New York, agreed entirely with Dr. Knapp in regard to the diphtheria bacillus. He did not think a diagnosis should ever be made of diphtheria without cultivation. Often even that is not sufficient and physiologic experiments are necessary. Within the last two weeks there was in the New York Eye and Ear Infirmary a case undoubtedly due to the Klebs-Loeffler bacillus. The smear showed absolutely nothing, but on cultivation the Klebs-Loeffler bacillus was found. There was no membrane. It was decided by Dr. Price, in whose service the case occurred, that it might be well to use antitoxin, and application was made to the Board of Health for it, but they did not get a satisfactory cultivation and refused the antitoxin. Except for the Klebs-Loeffler bacillus it is seldom necessary to make cultivation. It is a very easy matter, in his experience, to make a diagnosis of gonococcus; if there is any doubt, then, of course, a cultivation can be made. The pseudodiphtheria bacillus has caused him considerable trouble, and in nearly every case examined he has found at least a few so-called pseudo-bacilli. In two instances in which there was apparently nothing else present he lost the eyes. It seemed in these cases there must have been something else present. He has always been in the habit of advising against operation (in the presence of these pseudo-bacilli) if there is much secretion present, waiting until the conjunctiva completely clears up.

DR. JOHN E. WEEKS, New York, has seen a case of gonorrheal ophthalmia presented as a case of diphtheria of the conjunctiva by one of the best clinicians, and when a properly stained preparation was examined the diagnosis was cleared up at once. He has seen cases of gonorrheal conjunctivitis that were not more severe than the ordinary case of mucopurulent conjunctivitis. Microscopic examination, however, revealed the true nature of the infection. The clinical examination alone is not sufficient. In regard to the diphtheria bacillus, if the clinical aspect of the case is taken in connection with the findings of the microscope he considers that sufficient to enable one to make the diagnosis.

DR. E. L. MEIERHOF, New York, said that unless one is constantly cultivating this work his bacteriologic diagnosis is very uncertain. He thinks this work, after all, should be left to men who are constantly associated with it. It is surprising what different opinions come from men constantly doing this work. This question between the Klebs-Loeffler or the pseudodiphtheria bacillus is one in point. He recalled the case of a physician he treated, who had an appearance like that of trachoma. Diagnosis was made by two different men; one obtained the Klebs-Loeffler bacillus and the other the pseudobacillus. The patient got well ultimately, after a long time. Unless one is constantly doing bacteriologic work his examinations in many cases will be unsatisfactory.

DR. BROWN PUSEY, Chicago, protested against the idea of turning the examinations over to some one else. A large per cent. (probably 50 per cent.) of the inflammatory cases that come to the ophthalmologist are of infection of the conjunctiva and cornea, and the diagnosis, treatment and prognosis of these cases depends entirely on the bacteriologic examination; the clinical diagnosis is utterly unreliable. Such being the case, it behooves the man treating these cases to find out for himself the nature of the infectious agent.

DR. EDGAR S. THOMSON, New York, said that there was just one thing more that he would call attention to with regard to examinations for diagnostic purposes, and that was whether we make smears or culture that is not the whole question as to causal relations of the disease. The questions of relative virulence of the germ and immunity of the patient must be considered as well as the clinical picture. In a conjunctivitis in which a certain germ of known virulence is found to predominate it may be assumed with reasonable certainty that it is the cause of the disease, but in a mixed infection it is a difficult matter, even with cultures and smears, to tell which is the causative factor. The diphtheria bacilli and the pneumococci exist in the conjunctiva in a non-virulent state, and we must, therefore, consider the whole question in order to arrive at an intelligent opinion. For that reason he doubted many of the mild cases of gonorrheal conjunctivitis. He believed that when the gonococci are the causative factor, and of any virulence, they penetrate and there is a severe reaction.

HISTORY OF THE MASTOID OPERATION.

ITS SURGICAL ANATOMY, ETC.

FRANK ALLPORT, M.D.

Professor Clinical Ophthalmology and Otology, Northwestern University Medical School.

CHICAGO.

So much has been written concerning the mastoid and its associated organs, and the ground has been surveyed by so many leaders in otology, that it seems almost presumptuous to say anything further on the subject, unless distinctly new light can be cast on places that now seem more or less enveloped in darkness.

The last word, however, has not yet been uttered on mastoid and its allied surgery, and until it has, individual experience and observation may, nay must, assist us in the accumulation of future information and in the systematizing and clarifying of present knowledge.

Mastoid surgery, with such complicating features as